AD

Report Number 3

AD 740754

# FLUORESCENCE SPECTRA, POLARIZATION, AND LIFETIMES OF BIOLOGICAL COMPOUNDS IN LIVING CELLS AND IN MODEL SYSTEMS

ANNUAL REPORT

by

Charles N. Loeser

Department of Anatomy
School of Medicine
University of Connecticut Health Center
Farmington, Connecticut 06032

January 1972



Life Sciences Division
Army Research Office
3045 Columbia Pike
Arlington, Virginia 22204





22

Destroy this report when it is no longer needed. Do not return it to the originator.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

AD_____

Report Number 3

# FLUORESCENCE SPECTRA, POLARIZATION, AND LIFETIMES OF BIOLOGICAL COMPOUNDS IN LIVING CELLS AND IN MODEL SYSTEMS

ANNUAL REPORT

by

Charles N. Loeser

Department of Anatomy
School of Medicine
University of Connecticut Health Center
Farmington, Connecticut 06032

Grant/Contract No. DAHC19-71-G-0011

Department of the Army Project No. 2N061102B71D

January 1972

Life Sciences Division
Army Research Office
3045 Columbia Pike
Arlington, Virginia 22204

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

| 1. ORIGINATING ACTIVITY (Corporate author) | 2a. REPORT SECURITY CLASSIFICATION |
|---|---|
| The University of Connecticut Health Center<br>Farmington, Connecticut 06032 | 2b. GROUP |

3. REPORT TITLE

Fluorescence Spectra, Polarization and Lifetimes of Biological Compounds in Living Cells and Model Systems

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Third Annual Technical 1 Jan., 1971 - 31 Dec., 1971

5. AUTHOR(S) (First name, middle initial, last name)

Charles N. Loeser

| 6. REPORT DATE | 7a. TOTAL NO. OF PAGES | 7b. NO. OF REFS |
|---|---|---|
| January 1972 | 21 | 21 |

| 8a. CONTRACT OR GRANT NO. | 9a. ORIGINATOR'S REPORT NUMBER(S) |
|---|---|
| DAHC19-71-G-0011<br>b. PROJECT NO.<br>2NO61102B71D<br>c.<br>d. | 3<br>9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) |

10. DISTRIBUTION STATEMENT

Unlimited

| 11. SUPPLEMENTARY NOTES | 12. SPONSORING MILITARY ACTIVITY |
|---|---|
| None | Life Sciences Division<br>Army Research Office |

13. ABSTRACT

The purpose of this experimentation is to extract molecular information about the interaction between a variety of chemical substances of biologic interest with intact living cells studied at the microscope level if possible. The goal of the work is to seek the nature of in vivo complexing of compounds whose mechanism of binding is not fully understood and thus to clarify the effect of agents known to have biologic action, therapeutic or toxic.

By special instrumentation three parameters of fluorescence (spectrum, decay time, and polarization) are obtained from the complexes formed in the cells. The analysis of the three parameters can produce data detecting electronic energy transfer mechanisms, states of molecular orientation, molecular volume of complexes, certain degrees of local freedom (Brownian rotation characteristics) and the presence or absence of macromolecule formation.

This year's report, as in the case of the other reports, is arranged in alternating sections on instrumentation and on biologic experimentation.

Progress reported on instrumentation includes calibration and standardization of the optical bench for polarization of fluorescence and also modification of the TRW nanosecond decay time computer for the production of polarization data and nanosecond polarized fluorescent decay curves.

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Security Classification

## SUMMARY

The purpose of this experimentation is to extract molecular information about the interaction between a variety of chemical substances of biologic interest with intact living cells studied at the microscope level if possible. The goal of the work is to seek the nature of in vivo complexing of compounds whose mechanism of binding is not fully understood and thus to clarify the effect of agents known to have biologic action, therapeutic or toxic.

By special instrumentation three parameters of fluorescence (spectrum, decay time, and polarization) are obtained from the complexes formed in the cells. The analysis of the three parameters can produce data detecting electronic energy transfer mechanisms, states of molecular orientation, molecular volume of complexes, certain degrees of local freedom (Brownian rotation characteristics) and the presence or absence of macromolecule formation.

This year's report, as in the case of the other reports, is arranged in alternating sections on instrumentation and on biologic experimentation.

Progress reported on instrumentation includes calibration and standardization of the optical bench for polarization of fluorescence and also modification of the TRW nanosecond decay time computer for the production of polarization data and nanosecond polarized fluorescent decay curves.

Biologic experimentation is reported in three areas:

a. Antimalarial compounds. After considerable technical difficulties, it was possible to measure the polarization of quinacrine in single parasitized red blood cells. The data is preliminary but the figures appear to differ markedly from polarization measured in our standard ascites cell models.

b. ANS. As a pilot experiment for applications of fluorescent probes to study disease mechanisms, we introduced ANS into suspensions of pancreatic islet cells from toadfish. The purpose was to evolve a method of demonstrating changes in membrane structure after experimental alloxan treatment. The method worked in that it was possible to obtain the decay times and fluorescent polarization from isolated ANS stained islet cells, but the data is too preliminary and scant to judge for conclusions on membrane change.

In addition, this fluorescent probe did not prove by decay time measurements to be sensitive to membrane alterations after poisoning by iodoacetate or sodium cyanide, although gross denaturation with heat or alcohol did produce marked changes. Polarization has not been tested yet. Other probes may well be more sensitive to delicate intracellular alteration at the membrane level.

c. Polycyclic hydrocarbons. During this working period four more polycyclic hydrocarbons were added to the initial work on 3,4-benzpyrene reported in the other technical reports. All the carcinogenic hydrocarbons have significantly different decay times in normal cells than in malignant ones. Of the noncarcinogens, perylene showed no change in decay time from normal to malignant environment but pyrene did. Possible factors mediating these effects are discussed.

Two publications resulted during the working period of this report. Abstracts are included.

## FORWARD

During this working period the principal investigator gratefully acknowledges the capable research assistance on this project of:

Mrs. Ellen Clark, Research Assistant II
Miss Marjorie Maher, Student Labor, Research Assistant III
Mr. Harvyn Tarkmeel, Special Research Technician in Anatomy (electronics)

All the workers recognize with gratitude the innumerable administrative and secretarial tasks impeccably carried out for them by Miss Enid Prindle, secretary.

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Resources, National Academy of Sciences-National Research Council.

TABLE OF CONTENTS

# TEXT

## I. The Proposal

Full description of the objectives and methods of the research proposed can be found in the original application for research support, January 1, 1969, and in the Annual Technical Reports of January 1970 and 1971. A brief summary, however, follows for convenience of the reader.

Three parameters of fluorescence (spectrum, decay time, and polarization) are studied in a variety of chemical substances of biological interest while these substances are present in intact living cells. Comparisons are made with the same fluorescence parameters studied in model complexes in vitro. The biological substances studied on this project are chosen because their mechanism of binding in the living state is not fully understood.

The purpose of this experimentation is to extract molecular information from individual intact living cells. The analysis of the three parameters of fluorescence can produce data detecting electronic energy transfer mechanisms, state of molecular orientation, molecular volume of complexes, certain degrees of local freedom (Brownian rotation characteristics) and the presence or absence of macromolecule formation.

The basic purpose of the experimentation is to clarify the effects of agents known to have biologic action, therapeutic or toxic. The chemical reasons for resistance or lack of it in the complexing of certain compounds acting specifically at target tissues are sought, (antimalarials, for example). Also, by the use of one or more of the fluorescence parameters the development of a simple method for detection of molecular alteration in pathologic states may be possible.

## II. Background

### A. Spectra

In the study of fluorescence spectra, various types of complexing of the same fluorochrome can be detected by changes in peak intensities, ratios or wavelength positions. This investigator's former work was largely devoted to the study of such complexes in living cells (1, 2, 3). It was the limitations in sensitivity of such spectral measurements which led this investigator to develop methods for adding more fluorescence parameters to complement the spectral studies.

B. Polarization

A polarized exciting beam is absorbed only by those molecules oriented in a particular plane (coinciding with the direction of transitional moment). If the absorbing molecule is fixed, the emitted ray will be in the same plane. Depending upon the freedom of motion of the molecule, the resulting observed fluorescence will be only partly polarized. Due to the dependence of the phenomenon on Brownian characteristics, the observed polarization is a function of the size and shape of the molecule, viscosity and temperature of the environment, and the degree of freedom permitted by the binding state. Closely bound ligand molecules may show depolarization because of electronic energy transfer, and the distances and mutual orientation of such electronic oscillators may be calculated from depolarization data (4). Small fluorescent probes attached to larger molecules exhibit important changes in polarization (5). Mean harmonic relaxation times may be calculated--see section C below and references 6 to 9.

C. Decay Time

Decay time as here used is the mean duration of the excited state. Non-exponential decay times are indicative of energy transfer between molecules and are more likely to occur when quenching results from complex formations rather than from simple collisions (10).

The figure is also needed for the application of the Perrin-Weber equation (6-9) to our work. This expression relates observed polarization of fluorescence, the polarization of the same molecule rigidly bound, and decay time to the overall degree of flexibility of the molecule. Knowledge of this characteristic is extremely useful in interpreting binding sites for compounds in living systems.

D. Nanosecond Polarization Decay Time Measurement

Stryer (11), Wahl (12) and others have shown that the combining of measurement of decay time and fluorescence polarization is an elegant means of obtaining an instant readout on the molecular flexibility of a fluorochrome, and Chance has applied the method to the study of delicate structural changes on mitochondrial membranes during energization (13).

This combination of the two parameters to produce nanosecond decay time polarization data is a highly desirable goal for us since it will permit direct determination of molecular flexibility inside living cells and dynamic changes when they occur. Also, the data produced at different wavelengths may distinguish multiple binding sites of the same molecule inside a cell. This last possibility is of great importance for our work because of the complexity of the intracellular environment.

## III. Approach to the Problem

### A. General

The three fluorescence parameters are collected from optically isolated microscopic portions of single living cells containing a fluorochrome of interest in nontoxic concentration. Ideally, polarization is studied as a function of decay time and the rotational relaxation is read out. Collected separately, polarization and decay time are applied to Perrin's equation. Fluorescence spectra are scrutinized for changes.

Similar data is collected from test tube model systems combining the compound with cell components suspected to be involved in the interaction.

Interpretations are then made of the complexing behavior of the fluorochrome *in situ*.

### B. Specific Methods

#### 1. Spectra

The television fluorescence spectroscopic method utilized in this project has been developed and described fully by S.S. West and this investigator (1, 2).

#### 2. Polarization

In principle, fluorescence polarization is measured by the introduction of polarizer and analyzer in the optical bench of a fluorescence microscope.

Polarization (P) is given by:

$$P = \frac{F_{11} - F_{\perp}}{F_{11} + F_{\perp}}$$

where $F_{11}$ and $F_{\perp}$ are the fluorescence intensities measured with analyzer and polarizer oriented parallel and crossed respectively.

Readout from a high sensitivity Centronics VMP 13/44k photomultiplier tube is via a Keithley picoammeter and a Systron Donner Model 9000 digital voltmeter.

To produce the polarization values of fluorescing suspensions of cells (as reported by us for anilino-naphthalene sulfonic acid in reference 16) we adapted the TRW instrument. A polarizer film of type HNP'B was placed in front of the sample cuvette and another film, rotatable, was placed at right angles to the exciting light so that emitted fluorescence must pass through it on route to the photodetector.

3. Decay Time

Intracellular decay time measurement from suspensions of cells in test tubes is carried out using a nanosecond spectral source and decay time computer made by TRW, Inc. The principles of this apparatus have been described by Mackey and Pollack (14). We have adapted the light source of this apparatus and its readout mechanism to measurement of decay time through the microscope (15).

4. Nanosecond Polarization Decay Times

The TRW apparatus as presently equipped with polarizer in front of the sample cuvette and analyzer in front of the photodetector has been used for the pilot studies reported herein on polarized fluorescence decay time curves.

5. Biological Material and Techniques

Preparation of the mouse ascites tumor cells used as standard test objects in our laboratory has been described. In addition, cultured fibroblasts and liver cells shaken apart can be used (16).

For the malarial studies, mice infected with P. Berghei were obtained from I.I.T. Research Institute of Chicago through the cooperation of Dr. E. H. Eckermann of Walter Reed Army Medical Center and Dr. Morris King of the Institute. Pooled malarial blood for cuvette and microscope study was stained with $10^{-3}$ quinacrine and washed six times with saline by sequential centrifugation.

For the ANS work reported in this report, islet cells were dissected from stunned toadfish, minced finely, and spun gently in saline for 45 minutes. They were then filtered through cheese cloth, centrifuged and resuspended in nine volumes of saline. ANS was added in a ratio of three parts dye to one part cell suspension.

For the NaCN and iodoacetate work reported, the cells were treated as in a typical ANS staining experiment (16) but pretreated for 5 minutes with NaCN at $10^{-3}$ and iodoacetate at $10^{-2}$ M respectively, then washed by centrifugation.

Preparation of the cells exposed to the polycyclic hydrocarbons is described in reference 17.

## IV. Results

### A. Publications

In the period covered in this report two publications have been accepted and will appear shortly in Experimental Cell Research. Titles and abstracts are included below:

1) Loeser, C.N., Clark, E., Maher, M., and Tarkmeel, H. Measurement of fluorescence decay times in living cells.

This paper reports a simple technique for measuring fluorescence decay times of intracellular compounds. The data can be obtained from suspensions of living cells, or, using a microscope, from single cells. The method employs a TRW, Inc. nanosecond spectral source and decay time computer which have been used previously for measuring decay times of aqueous solutions. Ascites tumor cells, liver cells, fibroblasts, bacteria, and cell fractions, after incubation with a fluorochrome and appropriate washing, can be suspended in a cuvette (or in the case of single cells, placed on a microscope slide) and the fluorescent decay time can be read out digitally in nanoseconds. The instrument is most accurate where actual decay values are $> 2$ ns, and under these conditions reads to a precision of $\pm$ 0.5 ns in clear solutions, and $\pm$ 1.0 ns for intracellular work.

Results obtained using the fluorescent probes, anilinonaphthalene sulfonate (ANS), toluidinyl naphthalene sulfonate (TNS), 3,4-benzpyrene (BP), and 2-amino-naphthalene (2-AN) are presented as examples.

2) Loeser, C.N. and Clark, E. Intracellular fluorescence decay time of anilinonaphthalene sulfonate.

This paper presents the intracellular fluorescence decay time of the probe anilinonaphthalene sulfonic acid (ANS) and compares the results to certain ANS complexes in vitro. There is relatively constant decay time for intracellular ANS over a range of concentrations in the incubating medium compared with marked variation in results with the complex of ANS-bovine serum albumin in vitro when concentration of the probe is varied.

Calculation of the apparent rotational relaxation time from the Perrin equation, using ANS intracellular decay time and polarization data, gave a tentative value of circa 66 ns. By comparison with the results of ANS complexes with cell fractions and with certain lipids these data support the concept that intracellularly the compound may be largely membrane located with a portion of the molecule in the lipid phase.

Cells damaged by heating or alcohol show longer decay time than those which have taken up ANS in the living state. Suggestions for refinement of technique are included in the discussion.

B. Unpublished Work

1. Instrumental

a. Optical bench for polarization

At the beginning of this working period, we were still not satisfied with the performance of the optical bench for polarization of fluorescence and were also searching for standard substances on which to base our values. We attempted some work with single crystals of anthracene and pyrene but found the literature questionable as to exact figures on which to depend.

During the month of May Dr. Francis W. Morthland collaborated in our laboratory and ran exhaustive tests on all of the optical components of the bench. Extinction values (between crossed polarizer-analyzer) were poor with no lenses in place, and it became obvious that the limiting factor was not the previously suspected array of lenses in the system but rather the quality of the polarizers we had been using. We turned to polaroid type HNP'B plastic films which promptly gave us extinctions ranging from 1:150 to 1:250 with all lenses in place.

After some experimentation with a dansyl sulfonamide-carbonic anhydrase standard (which proved unstable, and the polarization values have to be extrapolated from Perrin plots) we settled on comparing our bench measurements of certain "old favorites" (fluorescein and rhodamine B in glycerol) with values for these substances reported in the literature. At excitation wavelength 436 mu polarization of $10^{-5}$ M fluorescein is reported by Weber (18) to be 0.41 while rhodamine B at $10^{-6}$ M is practically depolarized (0.0244). Our values range from 0.39 to 0.40 for fluorescein at the same concentration and wavelength and 0.054 to 0.0750 for rhodamine B.

There is some day to day variation due to slight variation of lens settings (the condenser aperture is focused in the back focal plane of the objective with a Bertrand lens and the light arc is centered with respect to that, etc.) and for each experiment we measure the standard, and correct when experimental results where indicated.

b. TRW modification for polarization

With the TRW nanosecond decay time apparatus modified for polarization as described in the section on Specific Methods, it is possible for us to now determine polarization values at different temperatures from the

same cuvette samples that we ordinarily use for decay time determination. This permits us to draw Perrin plots (1/p vs. T[deg]/n[poise]) and to extrapolate thereby to $P_o$ a value needed for the Perrin equation where

$$\left(\frac{1}{P} - \frac{1}{3}\right) = \left(\frac{1}{P_o} - \frac{1}{3}\right)\left(1 + \frac{3\tau}{\rho}\right)$$

Here $\tau$ is decay time in nanoseconds and P is rotational relaxation time.

Heretofore in our use of the equation we had to assume $P_o$ values published by other authors from test tube systems possibly quite different from our cellular systems especially in viscosity. For example, in our Technical Report #1, in estimating the rotational relaxation time of ANS from intracellular decay time we used the P value of 0.252 published by Udenfriend (5) for cell systems, and the $P_o$ value of a typical ANS-BSA complex which Weber reports as 0.40 (19). Calculation gave a rotational relaxation time of 35 nanoseconds.

This year with the instrumental modification we were able to collect our own figures and extrapolate from them to a better estimate of $P_o$ in cells. Use of the Perrin equation with these figures results in what is very likely a figure closer to reality for intracellular rotational relaxation time--66 nanoseconds. The data, an interpretation, and a discussion of the problems in the use of the equation in cell work appears in our most recent publication (20).

Use of the TRW equipment with the polarizer and analyzer in the display of nanosecond polarization decay curves is potentially of much value (see discussion) for direct readout of rotational relaxation time. However, the deconvolution of these curves is a problem not yet adequately worked out in the laboratory, but is presently under attack.

It is of passing interest to note that unless the electric vector of the exciting light from the TRW lamp was oriented for vertical excitation, observed polarization at 90 degrees was minimal.

2. Biological Experimentation

a. Antimalarials

A major goal of this project has been the study of the fluorescence parameters of antimalarial compounds in individual parasitized red blood cells. This year we succeeded for the first time in recording through the microscope the fluorescence polarization of quinacrine in parasites in single rbs's. This data although preliminary is interesting. The polarization is ca. 0.265, apparently markedly different from the figure obtained when quinacrine is in ascites cells. This, as reported in last year's technical report, is 0.152.

We also succeeded in recording fluorescence decay time from the quinacrine stained parasitized cells in cuvette using the TRW equipment. This experimentation recently performed was carried out against a nitrogen pulse standard with which we were not satisfied and thus must be repeated for calibration in order to report even preliminary values.

Thus far we have not succeeded in obtaining sufficient drug uptake of other antimalarials (chloroquin, for example) to produce enough intensity of fluorescence to record.

b. Further work on ANS

As a pilot experiment for the applicability of our new instrumentation and methods to the study of disease mechanisms, we cooperated with members of this Anatomy Department who are studying experimental alloxan diabetes in the toadfish. These investigators would like to be able to demonstrate whether changes in membrane structure occur as a result of alloxan damage to the beta cells of the Islets of Langerhans. We introduced $10^{-4}$ M ANS into isolated islet cells as described in Specific Methods and were able to record decay times and polarization, but the data is not conclusive as yet.

It may be that ANS is not the ideal probe for intracellular membrane alteration demonstration. Using this compound we were unable to demonstrate significant decay time changes after cell treatment with either NaCN or iodoacetate (although gross denaturation with heat or alcohol did produce marked changes). Curiously there was a marked enhancement of fluorescence intensity (a result opposite to that which would be expected from the studies of Chance on mitochondrial membranes under conditions of respiratory inhibition (13).

c. Polycyclic hydrocarbons

During this working period four more polycyclic hydrocarbons were added to the initial 3,4-benzpyrene work reported in the 1970 technical report. (The decay time of benzpyrene was 3 nanoseconds shorter in normal cells than in fully malignant ones. Polarization of emission was 0.)

The table (following page) presents the intracellular decay times of the compounds thus far studied.

All of the carcinogenic hydrocarbons have significantly different decay times in normal cells than in malignant ones. Of the noncarcinogens, perylene shows no change in decay time from normal to malignant environment but pyrene does.

## TABLE I

**Fluorescence Decay Time of Intracellular Carcinogenic vs. Noncarcinogenic Hydrocarbons in Tumor and Normal Cells**

| | Decay Time in Nanoseconds | |
|---|---|---|
| | Ascites Cells | Liver Cells |
| Benzpyrene[a] | 15.2 | 11.7 |
| Methylcholanthrene[a] | 25.8 | 17.5 |
| Dibenzanthracene[a] | 34.6 | 26.1 |
| Perylene[b] | 6.3 | 6.4 |
| Pyrene[b] | 198 | 169 |

[a]Carcinogenic

[b]Noncarcinogenic

## V. Discussion

### A. Instrument Development

The problem of the calibration of the optical bench for polarization seems to be solved as far as possible at the moment. The major limitation lies with the solidness of the bench itself, but improvement along these lines would require total reconstruction with vertical instead of horizontal alignment. The limitation may well be offset by the ease of manipulability of the open horizontal construction. The best and most reliable standards seem to be fluorescein and rhodamine B.

The modification of the TRW equipment for measurement of polarization of fluorescence is certainly a valuable one, and we are at work on a relatively simple deconvolution method to correct for the fall time of the lamp and to produce direct readout of rotational relaxation times. There will, however, continue to be limitations in the TRW equipment including the inherent operator error in the manual comparison of the two oscilloscope curves and more importantly, the sensitivity and response time limitations of the photomultiplier especially when measurements are made through the microscope.

We are still convinced that the ideal method for recording low light level decay time and nanosecond polarized decay curves is the technique of photon counting. It is our hope that we may be able to introduce this method onto our optical bench this year.

Investigators using flash systems such as the TRW for the study of polarization should note that such data can only be produced with the correct orientation of the electric vector of the exciting light; i.e., vertical with respect to the observed fluorescence. The matter is theoretically treated by Perrin (21) and the explanation was called to our attention by Dr. G. Weber (18).

### B. Biological Experimentation

#### 1. Antimalarials

In this area much remains to be done. We have succeeded in our goal only with the antimalarial quinacrine and the data here is only preliminary. Thus, further work must first establish statistical validity to the figures reported and major effort must also be put to the matter of obtaining sufficient drug uptake of other antimalarials so that we can record decay time and polarization from them.

The data collected thus far only from nonresistant strains must be compared with similar values taken from stained drug resistant parasites. Rotational relaxation times of the molecules calculated from this data may reveal delicate differences in internal environment provided by resistant versus nonresistant strains.

2. Membrane probes

Our work here must turn to other probes than ANS. There are a number of these which may be much more sensitive to internal structural changes. The development of fluorescence methods to detect minute morphologic alteration is of importance for the understanding of the action of a number of toxic agents of which our experimental tools (iodoacetate and cyanide) may be relatively gross examples.

It should be pointed out that funds and time from this ARO project will not be needed to further the pilot study on the islet of Langerhans cells, but the interested investigators will be able to use the equipment we have developed.

3. Hydrocarbons

As pointed out in previous technical reports, this project uses the fluorescence parameters of polycyclic hydrocarbons as models of compounds with high energy transfer capability more than for their interest as carcinogens. In a fluorescence study of 3,4-benzpyrene it was incidentally discovered by us in 1970 that the decay time in liver cells and fibroblasts (normal cells) was shorter by 3 nanoseconds than in fully malignant cells. The polarization of emission for intracellular benzpyrene was zero (17).

It was of profound interest therefore to enlarge the series and the data of Table I shows that the three carcinogens now studied all show the same phenomenon as far as decay time is concerned. Interestingly, perylene, the one noncarcinogen with a decay time in the range of the others (6 - 30 ns) shows no change whether in malignant or normal cells. Pyrene, also a noncarcinogen, shows a change but its decay time is very long. Firstly, there is a difficulty of accuracy in the use of the TRW instrument with either very long or very short decay times. It may be, however, that all the hydrocarbons do show some change of decay time from normal to malignant cells and the change becomes more readily detectable as the decay time is lengthened. The possibility also exists that pyrene with its extremely symmetrical structure has special properties.

One of the difficulties in obtaining a larger series is the fact that not all the hydrocarbons are taken up by liver cells or ascites cells in sufficient quantity to record by our apparatus. The introduction of photon counting should aid us in this respect.

Obviously, it will be important in the coming months to measure also the polarization from the members of the series of Table I in which thus far decay time has been measured.

The faster decay time in normal cells may be due to greater concentration of the substance in these cells with consequent shortening of the decay time by interligand energy transfer. However, the total lack of polarization found in benzpyrene from the intracellular environment may well be indicative of a greater freedom of motion and consequent greater collisional possibility for the carcinogen in the normal cell than in the malignant one.

Given the possible importance of single and multiple transient weak bonding (H-bonds, Van der Waals, etc.) in the interaction of extrinsic molecules with cellular macromolecules, and the special electronic properties of specific regions (for example, the "K" regions of carcinogens), collisional frequency may be very important in the mediation of a variety of effects and may bear not only on the nature of carcinogenesis but also on the nature of drug resistance or susceptibility to toxic agents in living tissues.

C. Future Work

Experimental areas to be stressed in the coming year include the possibility of introducing photon counting to improve our present methods of decay time and polarization measurement. With respect to biologic work, emphasis will be placed on completing the antimalarial work, on the use of fluorescent probes other than ANS, and on the completion of more decay time and polarization data on the hydrocarbons.

Fluorescence spectra must be collected this year on all of the compounds used in the past to complete the three major parameters to be measured on this project.

## VI. Literature Cited


1) West, S.S., C.N. Loeser and M.D. Schoenberg. I.R.E. Trans. on Med. Electronics, M.E. 7: 138-142, 1960.

2) Loeser, C.N. and S.S. West. Ann. N.Y. Acad. Sci. 97: 346-357, 1962.

3) Sloane, G.H.I. and C.N. Loeser. Cancer Research, 23: 1555-1565, 1963. Also: Murray, M.R., E. Peterson and C.N. Loeser. In: Morphological and Biochemical Correlates of Neural Activity, edited by Cohen and Snider. Paul Hoeber Co., New York, 1964.

4) Weber, G. and S.R. Anderson. Biochemistry 8: 361-377, 1969.

5) Udenfriend, S., P. Zaltzman-Nirenburg and G. Guroff. Arch. Biochem. and Biophys. 116: 261-270, 1966.

6) Perrin, F. J. Phys. Radium, 7: 1, 1936.

7) Weber, G. Adv. Protein Chem. 8: 415-459, 1953.

8) Memming, R. Z. Phys. Chem. 28: 168, 1961.

9) Tao, T. Biopolymers 8: 609, 1969.

10) Chen, R.F., G.G. Vurek and N. Alexander. Sci. 156: 949, 1967.

11) Stryer, L. Sci. 162: 526-533, 1968.

12) Wahl, M.P. Compte. Rend. 260: 6891, 1965.

13) Chance, B. Proc. Natl. Acad. Sci. 67: 560-571, 1970.

14) Mackey, R.C., S.A. Pollack and R.S. White. Rev. Sci. Inst. 36: 1715, 1965

15) Loeser, C.N., E. Clark, M. Maher and H. Tarkmeel. Exptl. Cell Res. In press.

16) Loeser, C.N., E. Clark, M. Maher and H. Tarkmeel. Arch. Biochem. Biophys. 141: 762-763, 1970.

17) Loeser, C.N. Proc. Am. Assoc. Cancer Res. 12: 100, 1971.

18) Weber, G. J. Opt. Soc. Am. 46: 962-970, 1956.

19) Weber, G. and Daniel. Biochem. 5: 1900-1907, 1966.

20) Loeser, C.N. and E. Clark. Exptl. Cell Res. In press.

21) Perrin, F. Annales de. Physique 12: 169, 1929.